# DESIGN AND DEVELOPMENT OF BROAD-SPECTRUM ANTIMICROBIAL PEPTIDES AS TOPICAL AGENT

SYNOPSIS OF THE THESIS

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## 1. Abstract of proposed work plan:

Antimicrobial peptides (AMPs) have gained more attention because of their broad-spectrum activities and different mechanisms of action compared to conventional antibiotics. The conventional antibiotics are losing their effectiveness due to growing antibiotic resistance. The fundamental structural properties like short size, positive net charge and hydrophobic nature of AMPs makes it more effective against broad range of microorganism. AMPs could serve as weapons against wide range of pathogens to protect animal and plant kingdom. The risk of resistance development in microbial populations is likely to be low because most of AMPs target microbial cell membranes. Although AMPs possess considerable topical application like oxidation and microbial degradation, their clinical and commercial development still have some limitations, such as potential toxicity, susceptibility to proteases, and high cost of peptide production. In order to overcome this limitation, reasonable focused research need to be carried out to evolve new AMPs and process to facilitate commercial production of peptide antibiotics.

## 2. Introduction

The use of Antibiotics as a magic bullet against various life threatening infections has profound impact from the development of first antibiotic penicillin [1-3]. over the period of time the high diversity of antibiotics molecules has been discovered from different sources. However, the emergence of multi-drug resistance bacteria is a serious threat to public health. In recent past biologically active antimicrobial peptides with multidimensional properties have been emerged from different sources such as plant, animal, mammala & microorganisms. These peptides are being focused since the last two decades due to their beneficial functionalities, namely antioxidative, antihypertensive, anti-inflammatory, immunomodulatory, and antimicrobial activity. The risk of antibiotic resistance is a major health issue [4] and hence researchers are paying more attention to Antimicrobial Peptides (AMPs). AMPs are short peptides having less than 50 amino acid, positive net charge and hydrophobic nature.

One of the driving forces behind the research into AMPs could be their broad-spectrum antimicrobial activity and low risk of resistance development. A combination of a well-defined

cationic region together with an imperfect hydrophobic surface area frequently seems to result in broad spectrum activity [5]. The cationic nature of AMPs selectively interacts with the negatively charged surfaces of microbial membranes and hydrophobic portions are responsible for the interaction with hydrophobic components of the membrane. The peptide- lipid specific interactions cause the peptide translocation across the membrane (figure 1), leading to the displacement of lipid [6,7]. Compared to this, mammalian surfaces differ in their composition, containing zwitterionic phospholipids, sphingomyelin and cholesterol, which allows AMPs to selectively target microbial membranes [8]. Transient pore formation due to AMP-membrane interaction, might increase membrane permeability to water, cations and anions, leading ultimately to loss of cytoplasmic membrane integrity and cell death [9].

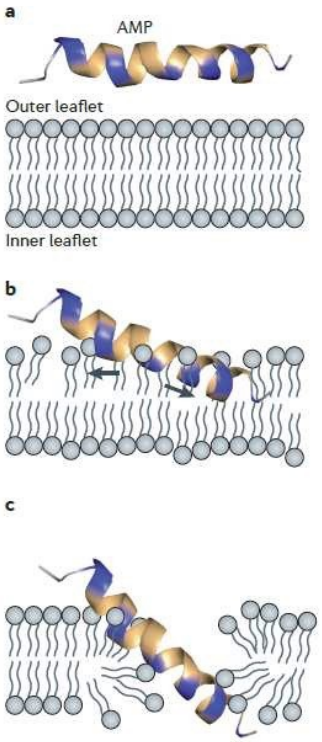


**Figure 1: Mechanism of membrane disruption and/or translocation by antimicrobial peptides.**

The antimicrobial peptide (AMP) is represented as a ribbon diagram, with positively charged residues indicated in blue (other residues are shown in yellow). a) AMPs selectively interact with the negatively charged surfaces of microbial membrane. b) Hydrophobic portions of AMPs further interacting with hydrophobic components of the membrane c) The peptide translocation across the membrane and displacement of lipid bilayer.

Initially proposed mechanism of action of AMPs was solely on the cell membrane. However several observations suggest that translocated peptides can alter cytoplasmic membrane septum formation, inhibit cell-wall synthesis, inhibit nucleic-acid synthesis, inhibit protein synthesis or inhibit enzymatic activity [10].

Antimicrobial peptides can be divided into four groups based on structural characteristics: α-helical, β-sheet, extended and loop peptides [11]. The classification of AMPs can be done on their mechanisms of action: membrane disruptors and non-membrane disruptors.

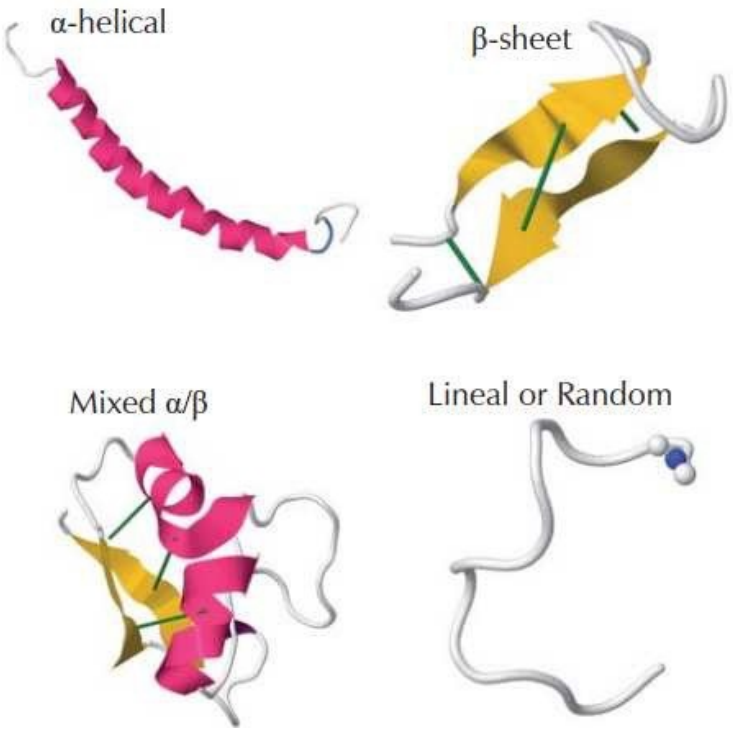


**Figure 2: AMPs structural characteristics: α-helical, β-sheet, mixed α/ β and lineal or random peptides**

**3. Review of Literature and Development in the subject:**

Many AMPs exhibit an extraordinarily broad range of antimicrobial activity covering both Gram-positive and Gram-negative bacteria as well as fungi, viruses, and unicellular protozoa [12]. Besides having a direct antimicrobial activity, several AMPs display ability to modulate the innate immune responses of the host and thereby indirectly promote pathogen clearance [13]. The widespread distribution and abundance of AMPs in all multicellular organisms underscores

their critical role in innate immunity. AMPs showed broad-spectrum antimicrobial activities against various microorganisms, including Gram-positive and Gram-negative bacteria, fungi, and viruses [14-15] Their importance is further demonstrated by the increased infection susceptibility of mice genetically modified to lack the gene encoding for the mouse analog of the human AMP LL-37 [16] and of humans with diseases associated with reduced AMP production such as atopic dermatitis [17]The bactericidal agents were isolated from Bacillus brevis and showed antimicrobial activity against Gram-positive bacteria [18]. The Magainins, a class of antimicrobial peptides from Xenopus skin was reported in 1987 [19].

The first reported animal-originated AMP is defensin, which was isolated from rabbit leukocytes in 1956 [20]. In the following years, bombinin from epithelia [21] and lactoferrin from cow milk [22] were both described. During the same time, it was also proven that human leukocytes contain AMPs in their lysosomes [23]. In addition to naturally existing AMPs, some synthetic AMPs were also used to treat biofilms. In a study by Okuda *et al.*[24], nisin A and lacticin Q were tested against mature biofilms of a MRSA strain, *S. aureus* MR23. In another study, GL13K derived from human parotid secretory protein (PSP) killed 99.9% of 24 hour biofilm cells of *P. aeruginosa* when it was added at 100 µg/mL for a two hour treatment [25]. NRC-16, a synthetic peptide, was tested against biofilm formation of three *P. aeruginosa* strains and compared with antibiotics such as ampicillin, chloramphenicol, and ciprofloxacin. NRC-16 showed biofilm inhibition at 8 µg/mL, which is 64 fold less than the antibiotic concentrations required to kill these *P. aeruginosa* strains [26]

**4. Objectives of Research Hypothesis:**

a) De-novo design of AMPs

b) Evaluation of antimicrobial activity of AMPs.

c) Method development for AMPs synthesis.

d) Characterization of AMPs.

## 5. Methodology:

Different strategies and physical factors will be considered while designing new anti-microbial peptides. The physiochemical properties such as size, charge, solubility, hydrophobicity are important for their antimicrobial activities and at the same time these factors will be considered alongside different strategies to combat their major hurdles and limitations including physical stability, toxicity, high production cost in development process. [27, 28]

The mathematical models will be used to infer quantifiable peptide properties including hydrophobicity and charge. Subsequently quantitative structure–activity relationship models (QSAR) will be used to screen out best AMP's. The powerful bioinformatic tools such as Swiss Model, Rosetta, HHpred, and I-Tasser to predict secondary structures may be explored in this study [29].

High production cost can be resolved by the production of smaller peptides with high stability. The major emphasis will be given on development of compatible expression hosts, for instance, E. Coli to reduce production cost [30]. Alternatively, the chemical synthesis of peptides can be carried out using classical solid-phase methods to facilitates the production of peptides which are difficult to express in bacteria [31]. The incorporation of unnatural or modified amino acid may be possible using this approach to reduce AMP's susceptibility to the proteases.

The *in vitro* antimicrobial activity of AMP's will be tested by disc diffusion assay, in this procedure, agar plates will be inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a different concentration will be placed on the agar surface. The antimicrobial agents are expected to diffuse into the agar and inhibit the growth of the test microorganism and then the diameters of inhibition growth zones will be measured [32].

For the determination of Minimum Inhibitory Concentration (MIC) values, dilution methods will be used. Two-fold dilutions of the antimicrobial agent (e.g. 1, 2, 4, 8, 16 and 32 mg/mL) in a liquid growth medium will be prepared and then, each tube will be inoculated with a microbial inoculum prepared in the same medium. After well-mixing, the inoculated tubes will be incubated under suitable conditions depending upon the test microorganism. The lowest

concentration of antimicrobial agent that will completely inhibit the growth of the organism in tubes detected by the unaided eye will be determined empirically [33]

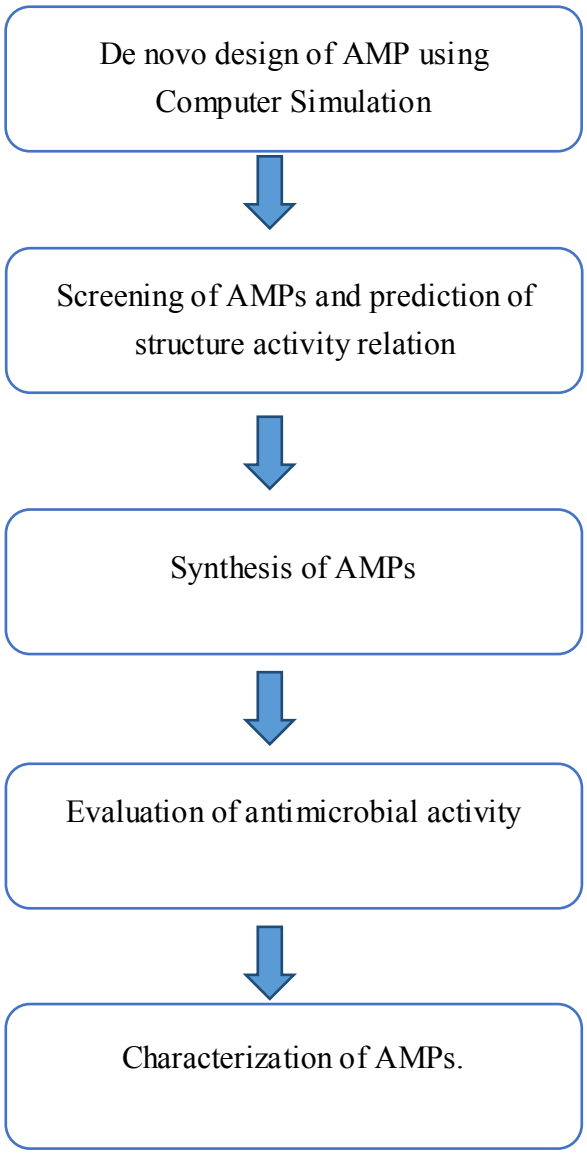
De novo design of AMP using Computer Simulation
Screening of AMPs and prediction of structure activity relation
Synthesis of AMPs
Evaluation of antimicrobial activity
Characterization of AMPs.

**Diagram: Depicting high level overview of workflow**

## 6. Proposed work plan

| Objectives | 1 Month | 6 Month | 12 Month | 18 Month | 24 Month | 30 Month | 36 Month |
|---|---|---|---|---|---|---|---|
| a) De-novo design of AMPs | | | | | | | |
| b) Evaluation of antimicrobial activity of AMPs. | | | | | | | |
| c) Method development for AMPs synthesis. | | | | | | | |
| d) Characterization of AMPs. | | | | | | | |
| e) Submission | | | | | | | |

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